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Assessment of predatory ability of native and non-native freshwater gammaridean species: A rapid test with water fleas as prey

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Abstract
Predation rate with relation to species, sex and water temperature was tested among four different gammaridean species: Dikerogammarus villosus, Gammarus roeselii, Gammarus pulex and Gammarus fossarum. Tests were performed in microcosms in climate-controlled rooms at five different temperatures. Daphnia magna, a common water flea, served as prey. On average D. villosus showed the highest consumption rate of Daphnia magna over the entire temperature range, followed in decreasing order by G. pulex, G. roeselii and G. fossarum. The predation rate of all species showed a distinct peak at 20°C. Correction of predation rates for body size gave somewhat different results. D. villosus is then still the most predatory of all gammaridean species tested followed by G. pulex, G. fossarum and G. roeselii. The outcome of the Daphnia tests is consistent with results of other studies with different prey. This supports that the Daphnia test is a good and quick indicator of the predatory abilities in gammaridean species at varying temperatures, and allows the prediction of how changing temperature regimes influence invasion impacts [Current Zoology 57 (6): 836–843, 2011].

Keywords Daphnia, Temperature, Predation rate, Amphipods, Invasions

Gammaridean amphipods have been considered as purely shredders, but are in fact omnivores that feed on a wide variety of food (Dick et al., 1997; Monakov, 2003; Felten et al., 2008; Platvoet et al., 2009b). They can exist in high densities and dominate the macroinvertebrate fauna in streams and rivers (Wesenberg Lund, 1939; Giller and Malmquist 1998). They can occupy prominent positions in local food webs, as they consume decaying material, detritus, soft macrophyte tissue, algae, other amphipods, their own offspring, small invertebrates, fish eggs, and fish larvae. They are in turn consumed by other macroinvertebrate species, fish and birds. Gammarideans can have high reproductive output and capacity (Kley and Maier, 2006; Grabowski et al., 2007; Bacela et al., 2009), and also have a varying ability in adapting to changing environments (Bruijs et al., 2001; Wijnhoven et al. 2003).

Gammarideans are one of the most successful invaders in freshwater ecosystems. They are often introduced in lakes and rivers to enhance fish stocks (see for a review Holdich and Pöckl, 2007). Successful invasions often coincide with changes in species abundance in the invaded waters: native gammaridean species start to decline or even become completely replaced by the new invading species (Leuven et al., 2009; Platvoet et al., 2009a). Mechanisms such as intraguild predation are supposed to be important when native gammaridean species become replaced by non-native ones (Kinzler and Maier, 2003; Dick, 2008). They can strongly influence the whole macroinvertebrate community through predation when they become very invasive (Kelly et al., 2006; Van Riel et al., 2006; Noordhuis et al., 2009).
Their role as a competitive predator in freshwater ecosystems can be dominant (Dick et al., 2002), but predatory behaviour and skills of various gammaridean species depend on many factors. These factors include differences in water temperature, morphology, morphological change, sex, body size, the amount and quality of available food (Van der Velde et al., 2009) and even parasites (Dick et al., 2010). It is clear that different gammaridean species show varied predatory characteristics and that there is a picking order in competition experiments with respect to intraguild predation (Dick et al., 1993; Dick and Platvoet, 1996, 2000; Van Riel et al., 2007, 2009; Platvoet et al., 2009a).

In this experiment we monitored the predation rate of four species, Dikerogammarus villosus, Gammarus roeselii, Gammarus pulex and Gammarus fossarum on water fleas with varying sex, size and water temperature. Predation rates may increase with larger individuals, male individuals, or those with strong invasive capabilities (Van der Velde et al., 2009). Based on earlier observations (Krisp and Maier, 2005; Bollache et al., 2008; Felten et al., 2008) it was hypothesized that G. villosus would be the most predatory and G. fossarum the least predatory species, with G. pulex and G. roeselii in between. The main research questions in this study are: a) are there differences in water flea predation rates between gammaridean species related to varying temperature, sex and size, b) are these differences species specific, and c) is a quick and simple screening test based on the consumption of water fleas a reliable assessment of their predatory capabilities so that we can predict the ecological impact of new and expanding gammaridean species compared with that of species already present?

1 Materials and Methods

In this experiment four gammaridean species were selected. Gammarus pulex and Gammarus fossarum are native species in the Netherlands, but G. pulex is an invader impacting ecosystems elsewhere including Ireland (Piscart et al., 2009; MacNeil et al., 2010). Gammarus roeselii is an early invader in the Netherlands most likely originating from the Balkan, extended its area to the Netherlands most likely through transport of water plants (Jazdewski, 1980), and is expanding in western Europe (Holdich and Pöckl, 2007). The most recently discovered invasive species used in this study is the Pontic-Caspian Dikerogammarus villosus, first found in 1995 in the Netherlands, which is still expanding over all of Europe (Casellato et al., 2007), including the British Isles (MacNeil et al., 2010).

1.1 Sampling locations

Gammarideans and site water for the experiments were collected in the Netherlands from October 2008–March 2009. Gammarus roeselii and Gammarus pulex were collected from a small stream named the Kroonbeek near Milsebeek (51°43′47″N, 5°58′11″E). Gammarus fossarum samples were collected from a small, shallow forest stream named Filosofenbeek flowing down from the glacial moraine hill, Duivelsberg, near Beck (51°49′12″N, 5°56′24″E). Dikerogammarus villosus samples were collected at a sluice near Weurt (51°51′00″N, 5°49′12″E). This sluice controls the water level of a canal that connects the river Maas (Meuse) with the river Waal (Maas-Waalkanaal). D. villosus was also sampled in the Gouwzee (52°28′11″N, 5°02′59″E) and at Van Ewijk’s sluice (52°52′48″N, 4°52′12″E). Water fleas Daphnia magna were bought from an aquarium shop to be used as prey within a few hours from purchase to be sure that they are alive and in good condition.

1.2 Storage

Gammarideans sampled from the field were kept in containers with water from their original environment at 20°C and were fed with decomposing plant material and small flocks of fish food (Tetramin). Individuals were taken from this stock for experiments.

1.3 Experimental design

The gammaridean species preyed on water fleas Daphnia magna at various constant temperatures (4°C; 12.5°C; 20°C; 25°C and 30°C) in climate controlled rooms. The tested gammaridean species are able to tolerate these temperatures (Wijnhoven et al., 2003; Van der Velde et al., 2009). Before each test, the gammarideans were stored overnight at the predetermined test temperature for 24 hours and were not fed.

Site water was then put in 20 of 40 rectangular, glass compartments (5×5 cm², height 2 cm) in a glass container. One individual of the study species was put in each filled compartment. Compartments were left empty in between individual tests to avoid unwanted influences of individuals with each other. We used five of these containers per species for every temperature, testing 100 individuals in this way for every temperature and species. After 30 minutes of acclimation for the amphipods in their container compartments, the water fleas were added. We used 20 water fleas per compartment (density: 400 individuals per litre), meaning 20 × 20 × 5 = 2000 fleas per species and temperature. The experiment was carried out at constant light conditions.
There was no flow in the compartments, but the water added was well aerated before. After the water fleas were added, the experiment lasted exactly one hour. The gammaridean amphipods were then removed from each container using a pair of pincers, and the number of remaining water fleas was counted in order to calculate the number of consumed water fleas per individual per hour. The gammarideans were transferred to numbered glass tubes with 70% ethanol to preserve them for sex determination in the laboratory using a stereomicroscope, and to measure their body length from anterior margin of the head to the posterior margin of the telson. The body lengths of the gammarideans tested ranged from 6–23 mm, viz. 4–14 mm for males, and 4–14 mm for females of G. fossarum, 7–18 mm for males, and 6–17 mm for females of G. pulex, 8–20 mm for males, and 7–19 mm for females of G. roeselii, and 9–23 mm for males, and 8–21 mm for females of D. villosus (Fig. 1).

At the end of the experiment, 20 × 5 × 5 × 4 (20 individuals in each of five containers for each of five temperatures and for each of four species) = 2000 feeding results (500 per species) were acquired. Predation rates (PR) were presented for every species, and males and females of each species. In order to determine the predation rate in relation to body length the number of consumed water fleas by an individual gammaridean amphipod was divided by the body length (mm) of the amphipod (PRC).

1.4 Statistics

The effects of species, sex and temperature on predation rate and predation rate corrected for body size of gammarideans were tested using an analysis of variance (ANOVA) and Games-Howell post hoc tests. All statistical analyses were performed with SPSS 15.0 for Windows®.

2 Results

In all experiments all gammaridean species showed a feeding response to the water fleas provided. The second antennae and gnathopods were used to catch and handle the fleas (Fig. 2). There was a clear difference between the feeding rates of the four gammaridean species. The number of consumed water fleas for each species in decreasing order was D. villosus > G. pulex > G. roeselii > G. fossarum (Table 1). D. villosus consumed 24.5 % of the water fleas offered, G. pulex 19.5 %, while G. roeselii and G. fossarum consumed 10.2 and 9.4 %, respectively. This sequence would be in the reverse order if the number of individuals per species that did not consume any water fleas was considered (Table 1). In all experiments individuals were observed that did not have consumed any water fleas. Of D. villosus 9.3 % of the specimens did not consume any water fleas, for G. pulex this figure was 10.9 %, and for G. fossarum and G. roeselii this value was 28.4 % and 29.9 %, respectively. Males of all species tested consumed more water fleas than females: D. villosus (male vs female) 26.9 vs 21.9 %, G. pulex 21.4 vs 16.5 %, G. roeselii 11.9 vs 7.6 % and G. fossarum 11.9 vs. 6.8 %. The percentages of males and females that had not consumed any water fleas was at an increasing order (males versus females): D. villosus 5.8 vs 13.1 %, G. pulex 7.7 vs 15.6 %, G. roeselii 25.2 vs 37.1 % and G. fossarum 18.2 vs 38.6 %.

After correction for body size, the order of species that consumed water fleas became D. villosus > G. pulex > G. fossarum > G. roeselii (Table 1).

Fig. 1  Size distribution (body length) of used specimens of A) G. fossarum, B) G. pulex, C) G. roeselii and D) D. villosus
Fig. 2 How *Dikerogammarus villosus* catches water fleas from the water column

A. *D. villosus* catches a water flea by a quick stroke of its antennae in particular the second antennae. B. The water flea is brought by these antennae towards the gnathopods (C) and hold by these near the mouth parts for consumption (D) (photos D. Platvoet).

The *P*-values of the ANOVA for temperature, species, sex and the interaction of temperature and species are much less than 0.05 (Table 2). We thus rejected the null hypothesis that there is no difference between predation frequencies sec or predation rates corrected for body size of the gammarideans. This means that temperature, species, sex and the interaction between changing temperature and species significantly affects predation rates.

Predation rates of gammarideans differed significantly in most pair wise comparisons of temperatures, except for 12.5°C vs 25°C and 30°C (Games-Howell post hoc test, *P*=0.071 and 0.999, respectively). When size-corrected, the consumption rate at different temperatures showed there were significant differences in results except at 12.5°C vs 30°C (Games-Howell post hoc test, *P*=0.781). The results show predation optimum curves for every species with lower feeding activities at

| Table 1 Data on the numbers of *Daphnia* consumed by the various gammaridean species and not consuming gammarideans during the tests |
| D. *villosus* (males, females) | G. *pulex* (males, females) | G. *roeselii* (males, females) | G. *fossarum* (males, females) |
| Number of consumed *Daphnia* | 1397, 1037 | 1229, 635 | 727, 293 | 587, 341 |
| Number of consumed *Daphnia/mm* (corrected for size) | 0.350, 0.334 | 0.324, 0.285 | 0.162, 0.120 | 0.258, 0.183 |
| Number of not consuming gammarideans | 15, 31 | 22, 30 | 77, 72 | 45, 96 |
| Range in number of *Daphnia* consumed | 0 – 9 0 – 7 | 0 – 6 0 – 6 | 0 – 3 0 – 1 | 0 – 7 0 – 4 |
| 4 °C: | 0 – 12 0 – 9 | 0 – 10 0 – 17 | 0 – 6 0 – 7 | 0 – 5 0 – 3 |
| 12.5 °C: | 0 – 16 0 – 13 | 0 – 17 0 – 12 | 0 – 12 0 – 10 | 0 – 11 0 – 6 |
| 20 °C: | 0 – 17 0 – 15 | 0 – 17 0 – 8 | 0 – 8 0 – 3 | 0 – 10 0 – 10 |
| 25 °C: | 0 – 14 0 – 15 | 0 – 15 0 – 11 | 0 – 15 0 – 6 | 0 – 6 0 – 4 |
| 30 °C: | 0 – 12 0 – 9 | 0 – 10 0 – 7 | 0 – 10 0 – 10 | 0 – 6 0 – 4 |

| Table 2 ANOVA-tests for effects of factor variables (temperature, species and sex) and their interactions on uncorrected and body size corrected predation rate of gammarideans species on water fleas |
| Source of variation | Dependent variable | df | *F* | *P* |
| Temperature | PR | 4 | 69.6 | <0.001* |
| | PRC | 4 | 83.0 | <0.001* |
| Species | PR | 3 | 163.0 | <0.001* |
| | PRC | 3 | 86.1 | <0.001* |
| Sex | PR | 1 | 51.3 | <0.001* |
| | PRC | 1 | 5.7 | 0.017* |
| Temperature * Species | PR | 12 | 8.0 | <0.001* |
| | PRC | 12 | 5.9 | <0.001* |
| Temperature * Sex | PR | 4 | 0.5 | 0.719 |
| | PRC | 4 | 1.4 | 0.217 |
| Species * Sex | PR | 3 | 0.8 | 0.477 |
| | PRC | 3 | 1.4 | 0.228 |
| Temperature * Species * Sex | PR | 12 | 1.6 | 0.094 |
| | PRC | 12 | 1.7 | 0.054 |

PR: Predation rate; PRC: Predation rate corrected for body size (= PR / mm). *: Level of significance *P* < 0.05.
4°C and 30°C and maximum consumption at 20°C (Fig. 4).
Predation rates of the various species differed significantly in most cases except G. fossarum vs. G. roeselii (Games-Howell post hoc test, $P=0.553$). Differences between the predation rates of the various species when corrected for size were significant (Games-Howell post hoc test, $P<0.05$). Predation rates of G. fossarum and G. roeselii on water fleas were clearly lower than that of D. villosus and G. pulex (Fig. 3a). D. villosus showed the widest optimum curve of all species tested. Optimum feeding rates at 20°C differed

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**Fig. 3** Predation rate of gammaridean species on water fleas at various temperatures
A. Mean predation frequency on water fleas (±SE) related to five different temperatures for G. fossarum, G. roeselii, G. pulex and D. villosus (males plus females). B. Idem for males. C. Idem for females. D. corrected for size (males plus females).

**Fig. 4** Total number of Daphnia consumed per hour by cumulating the numbers of the five subsequent tests
for the four species in decreasing order: *D. villosus* = *G. pulex* > *G. roeselii* > *G. fossarum*. This sequence and the course of the graphs did not differ when males and females were presented separately (Fig. 3b, c).

When the predation rates were corrected for body size, the difference in predation rate between *D. villosus* and *G. pulex* minimized at the lower temperatures tested but remained apparent at 25°C, at which *D. villosus* showed the highest predation rate, while *G. roeselii* showed the lowest of the four species (Fig. 3d). The general trend in all gammaridean species tested was that males consumed more water fleas than females (Table 1 and 2).

In all species, both males and females showed the lowest predation rates at 4°C. *D. villosus* individuals presented high predation rates (>10 water fleas) at temperatures above 12.5°C (males) and 20°C (females), for *G. pulex* this occurred in both sexes above 12.5°C, in *G. roeselii* above 20°C and in the case of *G. fossarum*, at 20 –25°C (males) and 25°C (females) (Table 1).

The five tests yielded similar results for each species at each temperature leading to a linear increase in water flea consumption when the numbers of the five trials of test containers were cumulated (Fig. 4).

### 3 Discussion

The results of the experiments show clearly that all gammaridean species tested consume water fleas, but in different amounts. The resulting water flea consumption for all species in decreasing order was *D. villosus* > *G. pulex* > *G. roeselii* > *G. fossarum*, which was expected based on other investigations on the predation capacity and diet of the gammaridean species used (Krisp and Maier, 2005; Bollache et al., 2008; Felten et al., 2008). After correction for size, the sequence of the four gammarideans with regard to their predatory ability remains the same with the exception of *G. fossarum* and *G. roeselii*, which change places in the order after correction. In three of the four tested gammaridean species, males consumed more water fleas than females, but in the case of *G. roeselii* there is hardly any difference in water flea consumption between the sexes. *G. fossarum* and *G. roeselii* showed also the highest numbers of individuals that did not consume any water fleas at all during the tests.

Based on these observations one should predict that the species with the highest predatory impact is *D. villosus*, followed by *G. pulex*, then *G. fossarum* and lastly *G. roeselii*. The water flea consumption in relation to the water temperatures shows a similar picture. Water flea consumption peaked in all species at 20°C. This pattern matches with earlier experiments carried out with *D. villosus* preying on *G. fossarum* (Van der Velde et al., 2009). *D. villosus* showed its superior predatory capacity over the entire temperature range, especially at high temperatures. Only at 4°C and 20°C did *G. pulex* reach the same predatory level as this species. *G. roeselii* and *G. fossarum* showed similar consumption levels, except after size correction, when *G. fossarum* showed more effective predator abilities.

These results are in accordance with the results of other studies using other prey. Bollache et al. (2008) carried out predation experiments with *D. villosus*, *G. duebeni celticus*, *G. roeselii* and *G. pulex* with *Asellus aquaticus* as prey in order to obtain response curves showing the relation between the amount of prey eaten and prey density (FR). They found a sequence FR of *D. villosus* > *G. duebeni celticus*/ *G. roeselii*/ *G. pulex*. It is not clear what temperature these experiments were carried out at, although 14°C is mentioned as the storage temperature. Krisp and Maier (2005) carried out 24 h consumption experiments with *D. villosus*, *Echinogammarus ischnus*, *G. pulex*, and *G. roeselii* with various macroinvertebrates as prey at 18°C. They found also a sequence of consumption on *Asellus aquaticus* as well as chironomids of *D. villosus* > *E. ischnus* > *G. pulex* > *G. roeselii*. They also concluded that *G. roeselii* is the weakest predator of the gammaridean species they tested. In mixed prey experiments, all species most frequently and easily preyed on chironomid larvae, as also found by Dick et al. (2002) for *D. villosus* and *G. duebeni*. Pacaud (1948) had used chironomids as prey in an experiment to compare the predation of damselfly larvae and *G. pulex* at varying temperatures. He found that predation for *G. pulex* gradually increased from 5°C to 28°C. Increased predation by the gammaridean species at the higher temperatures was also demonstrated by Van der Velde et al. (2009) in the case of *D. villosus* feeding on *G. fossarum*, and also by diet studies showing higher amounts of animal remnants in the digestive tracts of gammaridean amphipods in summer.

The present experimental design with water fleas as food seems to provide the quickest and most consistent results on the predatory capabilities of the gammaridean species, allowing comparison and prediction of their possible predatory impact on ecosystems. Similar experiments can be carried out with chironomid larvae or oligochaetes (*Tubifex*) as prey. The use of water fleas as prey has the advantage of clear food units that were consumed completely allowing the predation rate to be
easily counted. With the exception of *G. fossarum* inhabiting fast flowing water in the upper reaches of streams, the other gammaridean species used in the experiments can exist in still water where water fleas can also develop their populations. Nearly all our tested gammaridean specimens were caught in wintertime in flowing water where water fleas are absent so that the specimens did not have any experience with catching water fleas. The different abilities to catch water fleas were thus based solely on a combination of morphology of the gammarideans and water temperature.

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